Short Term Scientific Mission, COST Action CA15134 Synergy for preventing damaging behaviour in group housed pigs and laying hens (GroupHouseNet)

By

Elske de Haas, postdoctoral researcher from Wageningen University and Research Centre, The Netherlands

at the

Avian Biology Group, Linköping University, Linköping, Sweden

From: 19th of March 2018 until the 5th of April 2018

Title:

Gene expression in the thalamus of chickens selected divergently on feather pecking

Background of the research

Feather pecking in chickens can be a form of behaviour, i.e. a behaviour which occurs in order to cope with a thwarted environment. The thalamus is an important regulator of emotion related behaviour, such as stress responses. As such it can relate to coping with stress and be influences by genetic preprograming of gene-expression in the thalamus. As feather pecking is a behaviour which involves group housed chickens, the COST Action CA15134 Synergy for preventing damaging behaviour in group housed pigs and laying hens (GroupHouseNet) has enabled to support me in doing a Short Term Scientific Research researching gene-expression in chickens at the University of Linköping, Sweden under the supervision of Prof. Per Jensen and Dr. Carlos Bosanga from 19th of March until the 5th of April, 2018.

Introduction

Neurotransmitter receptors play an important role in the neurochemistry of the brain. The activity of these receptors tune how much of a certain neurotransmitter is released, and how much is bound. In interaction with transporters they determine the activity of the system. Regarding feather pecking, evidence has been mounting in regards to the serotonergic system and dopaminergic system. These systems interact with the stress system, and modulate one another. Receptors of the serotonergic and dopaminergic system appear altered in chickens which feather peck (van Hierden et al., 2004; Flisikowski et al., 2010) as compared to control chickens. Therefore, in this Short Term Scientific Mission (STSM) I looked specifically at genes which transcribe proteins which influence the activity of receptors of the serotonergic and dopaminergic system, and at the precursors of these, as well as to glucocorticoid receptors which are important in the stress response system. For an overview of the genes studied in this STSM, see Table 1. For detailed description of primers see https://www.ncbi.nlm.nih.gov/gene.

Methodology

As behaviour can be an indication of a change in activity of gene expression, I looked at day old chicks which did not perform feather pecking behaviour and 8 week old chicks, which differed in feather pecking. These animals came from a selection experiment where founder birds were selected on bouts of gentle and severe feather pecking (Kjaer and Sorensen, 2002). Hereby they created a high feather pecking line (HFP) and a low feather pecking line (LFP). The animals of this STSM were of the 14th generation of selection, although parental birds were not selected anymore and a continuation of the lines was proceeded instead. In the parental animals the most severe feather peckers were, however, excluded as part of another project. The parental birds therefore contained only victims-peckers and victims, or neutrals but not perpetrators. Nevertheless, parental birds differed significantly in feather damage due to pecking (van der Eijk, personal communication). Both age groups (day old chicks, and 8 week old pullets) had sample size of 10 (5 HFP and 5 LFP). Chicks were sacrificed by decapitation, as

part of another sampling experiment. Brains were dissected in 7 parts, including the thalamus. Brains were stored in -80C upon further processing. First DNA and RNA was extracted by means of the D-Neasy Blood and Tissue Kit. cDNA was extracted from the RNA by means of a cDNA Maxima cDNA synthesis kit with Dnase. Quantity of RNA was assessed by Nanodrop assessment looking at the 230/280 ratio and 230/260 ratio. Bioanalyzer was used for quality control of the RNA. Primers of all genes were evaluated prior to use by colleagues of the Avian Group, Linköping University, Sweden. Two housekeeper genes were used to compare values of the gene expression with. These housekeeper genes were POL-2 and MG, as previously been validated by Amir Fallahdhahroudi of the Avian Biology Group. All samples were analysed with qPCR method in duplicate with housekeepers gene on each 96 well plate. Seven plates were used.

Statistics

Data was analysed by SAS 12.1. For each gene, expression levels were compared to the housekeeper gene POL-2 to validate increased or decreased expression. Differences between lines were assessed with an ANOVA testing the effect of line and age and their interaction. Only for TH 8-week old animals were compared while all other genes are compared between both ages, this was due to plate and time restriction.

Table 1. Genes and their description* studied in relation to feather pecking in chicken

Serotonergic system		**Dopaminergic system**		**Stress system**	
TPH1	**Tryptophan hydroxylase 1** This gene encodes a member of the aromatic amino acid hydroxylase family. The encoded protein catalyses the first and rate limiting step in the biosynthesis of serotonin; 5-hydroxytryptamine (5-HT).	TH	**Tyrosine hydroxylase** The protein encoded by this gene is involved in the conversion of tyrosine to dopamine. It is the rate-limiting enzyme in the synthesis of catechol amines, hence plays a key role in the physiology of adrenergic neurons.	NR3C1 or GR	**Nuclear receptor subfamily 3 group C member 1 – (GR)** This gene encodes glucocorticoid receptor, which can function both as a transcription factor that binds to glucocorticoid response elements in the promoters of glucocorticoid responsive genes to activate their transcription, and as a regulator of other transcription factors. This receptor is typically found in the cytoplasm, but upon ligand binding, is transported into the nucleus.
HTR 1a	**5-hydroxytryptamine receptor 1A** This gene encodes a G protein-coupled receptor for 5-HT, and belongs to the 5-HT receptor subfamily.	DR D1	**Dopamine receptor D1** This gene encodes the D1 subtype of the dopamine receptor. The D1 subtype is the most abundant dopamine receptor in the central nervous system. This G-protein coupled receptor stimulates adenylyl cyclase and activates cyclic AMP-dependent protein kinases.	NR2C1 or MR	**Nuclear receptor subfamily 2, group C, member 1 - (MR)** This gene encodes a nuclear hormone receptor characterized by a highly conserved DNA binding domain (DBD), a variable hinge region, and a carboxyl-terminal ligand binding domain (LBD) that is typical for all members of the steroid/thyroid hormone receptor superfamily. This protein also belongs to a large family of ligand-inducible transcription factors that regulate gene expression by binding to specific DNA sequences within promoters of target genes.
HTR 1b	**5-hydroxytryptamine receptor 1B** The protein encoded by this intron less gene is a G-protein coupled receptor for 5-HT. Ligand binding activates second messengers that inhibit the activity of adenylate cyclase and manage the release of serotonin, dopamine,	DR D2 - NI	**Dopamine receptor D2** This gene encodes the D2 subtype of the dopamine receptor. This G-protein coupled receptor inhibits adenylyl cyclase activity.	CHRH1	**Corticotrophin-releasing hormone receptor 1** This gene encodes a G-protein coupled receptor that binds neuropeptides of the corticotrophin releasing hormone family that are major regulators of the hypothalamic-pituitary-adrenal pathway. The encoded protein is essential for the activation of signal transduction pathways that regulate diverse physiological processes including stress.

	and acetylcholine in the brain.				
HTR 2a	**5-hydroxytryptamine receptor 2A** This gene encodes one of the several different receptors for 5-HT, that belongs to the G-protein coupled receptor 1 family.	DR D3 - NI	**Dopamine receptor D3** This gene encodes the D3 subtype of the five (D1-D5) dopamine receptors. The activity of the D3 subtype receptor is mediated by G proteins which inhibit adenylyl cyclase. This receptor is localized to the limbic areas of the brain.	CRHR2	**Corticotrophin-releasing hormone receptor 2** The protein encoded by this gene belongs to the G-protein coupled receptor 2 family, and the subfamily of corticotrophin releasing hormone receptor. This receptor shows high affinity for corticotrophin releasing hormone (CRH), and also binds CRH-related peptides such as urocortin. CRH is synthesized in the hypothalamus, and plays an important role in coordinating the endocrine, autonomic, and behavioural responses to stress and immune challenge.
HTR 2c	**5-hydroxytryptamine receptor 2B** This gene encodes one of the several different receptors for 5-HT, that belongs to the G-protein coupled receptor 1 family.	DR D4 - NI	**Dopamine receptor D4** This gene encodes the D4 subtype of the dopamine receptor. The D4 subtype is a G-protein coupled receptor which inhibits adenylyl cyclase.	CRH	**Corticotrophin releasing hormone** This gene encodes a member of the corticotrophin-releasing factor family. The encoded preproprotein is proteolytically processed to generate the mature neuropeptide hormone. In response to stress, this hormone is secreted by the paraventricular nucleus (PVN) of the hypothalamus, binds to corticotrophin releasing hormone receptors and stimulates the release of adrenocorticotropic hormone from the pituitary gland.
SLC6 A4 – or SERT	**Serotonin transporter** This gene encodes an integral membrane protein that transports 5-HT from synaptic spaces into presynaptic neurons. The encoded protein terminates the action of 5-HT and recycles it in a sodium-dependent manner. A repeat length polymorphism in the promoter of this gene has been shown to affect the rate of 5-HT uptake.				

* the description is based on human genes https://www.ncbi.nlm.nih.gov/gene

NI = not included due to not working primer of the gene

Results

No differences in gene expression in any of the genes were found between HFP and LFP chicks. Neither effects of age or in interaction with line effects were found in any of the genes studied. See table 2 for differences in expression between 1 day old HFP chicks (high feather pecking line) and LFP chicks (low feather pecking line) and between 8 week old HFP and LFP pullets. Table 2 is the expression in relation to housekeeper gene POL-2.

Table 2 Gene expression in relation to housekeeper gene PO2 of chicks of a high feather pecking (HFP) line versus chicks of a low feather pecking (LFP) line

HFP and LFP lines at 1 day of age and at weeks old					
	1 day old		**8 week old**		
Genes	**HFP**	**LFP**	**HFP**	**LFP**	**Anova (df) P value**
TPH1	8.23±0.56	7.20±0.53	6.90±0.27	7.68±0.42	F (1,17) = 0.03, P = 0.85
HTR1a	2.32±0.65	1.69±0.29	2.34±0.42	2.61±0.51	F (1,17) = 0.08, P = 0.78
HTR1b	-1.45±0.23	-2.12±0.37	-2.01±0.05	-1.39±0.35	F (1,17) = 0.00, P = 0.99
HTR2a	3.35±0.46	3.34±0.41	3.06±0.26	3.87±0.49	F (1,17) = 0.66, P = 0.43
HTR2b	2.27±0.09	2.48±0.46	2.46±0.37	3.37±0.32	F (1,17) = 2.22, P = 0.15
HTR2c	2.89±0.23	2.83±0.20	3.04±0.07	3.88±0.44	F (1,17) = 0.89, P = 0.36
SERT	4.16±1.42	4.63±1.60	2.94±0.66	4.60±1.40	F (1,17) = 1.01, P = 0.33
TH	ND	ND	0.94±1.03	0.57±1.98	F (1,17) = 0.18, P = 0.68
DRD1	2.66±0.45	3.27±0.30	3.26±0.13	3.64±0.54	F (1,17) = 1.06, P = 0.32
DRD2	10.61±0.54	5.85±4.02	9.05±0.61	9.40±0.57	F (1,17) = 1.16, P = 0.30
GR	0.56±0.24	-0.27±0.31	-0.05±0.28	0.05±0.16	F (1,17) = 1.11, P = 0.31
MR	0.57±0.27	0.08±0.33	0.41±0.26	0.83±0.28	F (1,17) = 0.00, P = 0.99
CHRH1	2.11±0.32	2.16±0.36	2.26±0.44	3.00±0.54	F (1,17) = 0.60, P = 0.45
CRHR2	1.79±0.38	1.33±0.40	2.55±0.09	2.51±0.29	F (1,17) = 0.39, P = 0.54
CRH	0.82±0.39	-0.18±0.32	0.90±0.23	0.63±0.54	F (1,17) = 1.54, P = 0.23

ND = not determined

Conclusion

This preliminary study on gene expression in HFP and LFP chicks at one day of age and at 8 week old did not reveal any line effects in regards to genes of the serotonergic, dopaminergic and glucocorticoid system. However, this study contained per age group only 5 animals, which is a relatively small sample size. To increase the power of the study more animals should be sampled and analysed for gene expression differences to give an exclusive picture of differences in gene expression. Interestingly these animals also came from different fathers, and it would be furthermore relevant to extent the data set to more animals so as to take into account the effect of paternal origin. Although no difference was found between ages in gene expression it would be interesting to include behavioural data of the 8-week old chicks to assess individual differences in gene expression in relation to their behaviour. As this data exists, a follow up analysis of gene-expression with behavioural data can take place. I would stress that to give a full conclusion, this data set should be extended to at least 6-8 animals per group to give an exclusive answer to whether gene expression differences in HFP and LFP chicks exist.

Acknowledgement

Gratefully acknowledged are Professor Per Jensen, Dr. Carlos Bosanga, Dr. Amir Fallahdhahroudi and Dr. Robin Abbey-Ly. Thanks to the COST Action CA15134 Synergy for preventing damaging behaviour in group housed pigs and laying hens (GroupHouseNet) I was able to set up this experiment on gene-expression in chickens of a high feather pecking line and low feather pecking line.